Background

Manganese (Mn) is an essential dietary element biologically utilized for numerous physiologic processes as a constituent of enzymes and an activator of other enzymes (Nielsen, 1999), such as Mn superoxide dismutase (MnSOD), a principal antioxidant enzyme of mitochondria (Leach and Harris, 1997). Mn enzymes are also involved in bone development (Keen and Zidenberg-Cherr, 1996), wound healing (Shetlar and Shetlar, 1994), and the metabolism of carbohydrates, amino acids, and cholesterol (Institute of Medicine 2001). Upon ingestion, Mn is subject to tight homeostatic control (Papavasiliou et al.,1966). Inhaled Mn, however, can bypass biliary excretion mechanisms and directly enter the systemic circulation (Davis, 1998; Davis, 1999; Keen et al., 1999). Inhaled Mn exposure may have a dose-related continuum of nervous system dysfunction, and the lowest level linked with neurotoxicity is unknown (Mergler et al., 1999). At the high end of exposure (>1 mg/m^3), Mn can result in Manganism, an extrapyramidal movement disorder similar to, but clinically distinguishable from, Parkinson's disease (Aschner et al., 2005; Huang et al., 1993; Pal et al., 1999). At concentrations near or below the reference concentration (RfC) of 50 ng/m^3 established by the U.S. Environmental Protection Agency (EPA, 2009), Mn neurotoxicity includes effects on neuropsychological and motor function (Mergler et al., 1999), postural stability (Hudnell, 1999; Standridge et al., 2008; Hernández-Bonilla et al 2011), and increased risk for physician diagnosis of Parkinson's disease (Finkelstein and Jerrett, 2007). Children and infants may be particularly susceptible for the neurotoxic effects of environmental ambient Mn exposure due to the development of the brain and central nervous system and the ability of ambient Mn to cross the blood-brain barrier and accumulate in the brain (Elder et al., 2006; Aschner, 1999; Aschner, 2000; Aschner, 2006). *In utero* exposure to environmental Mn has also been associated with decreased neurocognitive and neuromotor function in children (Takser et al., 2004).

Mn is frequently a component of ambient particulate matter (PM), a complex mixture of acids, organic chemicals, metals and soil or dust particles. The coarse fraction ($PM_{2.5-10}$) is often dominated by crustal materials (e.g., windblown dust, resuspension of road dust, demolition of buildings), whereas the particles in fine ($PM_{2.5}$) and ultrafine ($PM_{0.1}$) fractions are mostly formed through combustion processes including metal refining. Fine and ultrafine particles have been associated with adverse health effects (Schwartz et al., 1996; Pope et al., 2009) which can be attributed to the properties of these particles including size, surface area, and composition.

Eramet Marietta, Inc. (EMI), a ferromanganese refinery in operation since 1952 (Eramet Marietta, 2011) is located in Marietta, Ohio, a rural Appalachian American community situated along the Ohio River. The refinery has reported their fugitive Mn emissions to the EPA varying from 580,213 lbs/year in 1999 to 101,503 lbs/year in 2009 (EPA, 2011). An academic-community partnership was developed (Haynes et al., 2011) between the University of Cincinnati and the community to conduct an epidemiologic investigation of the neurobehavioral effects of Mn on children, the Marietta Community Actively Researching Exposure Study (CARES). The purpose of this study was to address, in part, one of the community-driven concerns of the “microenvironments” of children residing near the refinery by examining personal exposure to $PM_{2.5}$ and its constituents in a subset of the cohort.

Traditionally, exposure to particulate matter and Mn, in particular, in the ambient environment is measured by stationary outdoor air monitoring; however, outdoor stationary monitors may not be representative of personal exposures for several reasons. Stationary monitors are typically placed in locations far above the typical breathing zone, i.e., rooftops, and humans spend the majority of their time indoors (Klepeis et al., 2001). Furthermore, personal exposures are often higher than shown by microenvironmental measurements in indoor or

outdoor air because of so-called “personal cloud” effect, caused by closer proximity to pollution sources or particle resuspension due to human activity (Wallace, 2000). Thus, the goal of our study was to characterize personal exposure to Mn and PM2.5 in a cohort of children ages 7-9 years residing near a ferromanganese refinery. We also wanted to address the following research questions: 1) What is the relationship between personal air Mn, stationary air Mn, and time-weighted distance from the refinery (TWD) and 2) what is the relationship among biological Mn concentrations (hair and blood) and personal exposure to ambient Mn.

Methods

Study Population. Children ages 7, 8, and 9 years were recruited for participation in the personal air sampling through their enrollment in CARES. Eligibility for CARES includes children ages 7-9 who have resided in the Marietta area throughout their life and their biological mother having resided in the area during pregnancy with the child. Children were recruited for participation in CARES through recruitment letters sent home through schools, radio and newspaper ads, and fliers. Children enrolled in CARES from Marietta were selected to participate in the personal air sampling study if they reported residing in a non-smoking household. The study was approved by the Institutional Review Board of the University of Cincinnati and all participants signed an informed consent and assent.

Stationary Air Sampling. A stationary air sampler was positioned on the rooftop of a Marietta College building approximately 8 kilometers from EMI. The site was selected based on its high population density within the catchment area. Positioning the stationary air monitor in the highest population density area within the community provided an exposure scenario experienced by the anticipated majority of the study participants. Stationary samples were collected using Harvard-type $PM_{2.5}$ impactors (MS&T Area Sampler; Air Diagnostics, Inc,

Harrison, ME) with a high volume sampling pump, calibrated to 10 liters per minute (LPM) ± 0.5 LPM. The sampler was equipped with a sampler ring, a Whatman support pad to prevent filter blow out, and a 37 mm pre-weighed Teflon membrane filter (2 µm pore size; SKC Inc., Eighty-Four, PA) to collect airborne particles smaller than 2.5 µm. The sampling times were 48 hours ±2 hours. Three 48-hour samples were collected each week. Stationary air sampling was conducted over a 2-year time frame from October 2008 – September 2010. Only the results from the stationary air sampling during the time of the personal air sampling are used in these analyses.

Personal air sampling. Personal air samples were collected using a Personal Modular Impactor, PMI coarse (SKC Inc.). The PMI coarse sampler is a two stage impactor, capable of sampling for $PM_{2.5}$, $PM_{2.5-10}$, or both. The PMI coarse sampler was selected to minimize the bounce of large particles on the $PM_{2.5}$ filter by using an oiled impaction plate on the $PM_{2.5-10}$ stage instead of a collection filter.

Air pumps and battery packs were placed in a backpack, with a short Tygon® tube to connect the PMI sampler to the air sampling pump. The pumps were calibrated to 3 LPM ±0.1 LPM. The PMI sampler was equipped with the same type of Teflon filters used for outdoor sampling. The air sampling technician demonstrated how to wear the backpack and the sampler. The technician instructed to keep the air sampler near the child's breathing zone during times when the child could not comfortably wear the backpack, for instance during sleep the equipment could be laid on a nightstand. The sampling time was 48 hours ± 2 hours and the sampler remained in or near the child's breathing zone for the duration of the sampling time. All personal samples were collected during the Spring season of two consecutive years: April-June, 2009 and March–June, 2010.

During a home visit with the participating families, a study team member conducted a brief questionnaire to determine the child's potential exposure to particle sources during the sampling period. Questions included the child's exposure to cigarette smoke, use of heating and cooking devices, candle use, and time sampling setup was not with subject or was outside of the Marietta area. Throughout the 48 hour period, parents of participants filled out an activity log to indicate the activities of the child during the sampling period and to identify when and if the sampling device was not with the child during the sampling period.

Analysis of Air Sampling Filters. Teflon filters for personal and stationary outdoor samples were analyzed for particle mass gravimetrically and for Mn in the $PM_{2.5}$ fraction using a Thermo X Series II inductively coupled plasma mass spectrometer (ICP-MS) by a commercial laboratory (Research Triangle Institute, NC). Results were reported in $\mu g/m^3$ ($PM_{2.5}$ mass) and ng/m^3 (Mn). Ten percent (10%) of the samples were laboratory blanks and another 10% were field blanks to protect the validity of the sample results. The detection limits were 4.1 µg/sample for $PM_{2.5}$ mass and 2.5 ng/sample for Mn.

Time Weighted Distance (TWD). TWD was calculated based on time spent at home ($Time_{Home}$) and school ($Time_{School}$) and the distance of each ($Distance_{Home}$ and $Distance_{School}$) from the ferromanganese refinery divided by the child's total sampling time (48±2hrs). The TWD was calculated using the following equation:

$$TWD = \frac{(Time_{Home} * Distance_{Home}) + (Time_{School} * Distance_{School})}{Total\ Time\ Sampled} \quad (1)$$

Wind index. In order to account for the influence of wind direction on sampled concentrations of Mn, a continuous measure of the location of the child's home relative to the wind direction during the 48 hours of sampling and the location of the primary Mn emission source, EMI, Inc. was derived. This measure, referred to as the *wind index*, was calculated as

$$Wind\ Index = \frac{1-Cos(Angle_{Home_{Plant}} - Angle_{Wind})}{2} \quad (2)$$

where $Angle_{HomePlant}$ is the Euclidean direction of the Mn point source from the child's home and $Angle_{Wind}$ is the average wind direction during the 48-hour sampling period obtained from the National Climatic Data Center climate data from the Parkersburg, West Virginia at the Mid-Ohio Valley Airport. The cosine transformation allows for a rescaling of the difference in the home angle to the Mn point source and wind direction to a scale with range 0 to 1. Hence, a home directly upwind of the Mn point source during sampling will have a wind index variable of 0 and homes directly downwind a value of 1.

Biological Collection and Analysis.

Blood. Venipuncture samples were collected by trained phlebotomists using a 23 gauge butterfly needle and vacutainer cuff or needle and syringe. Proper preparation and blood collection techniques were employed to ensure that samples were not contaminated. Blood collection equipment and supplies were tested to be trace-metal free and were stored and set up in a manner that kept them free of dust and contaminants. Blood collection tubes were placed in a small bag after the box was opened to protect the tops from being contaminated. Kimwipes®, a lint free cloth, were used to cover the tubes, place blood collection supplies on, or used whenever needed to protect equipment from contamination.

The blood puncture area at the site of the antecubital vein was prepared by briskly scrubbing the area twice with alcohol pads in a circular fashion inside to out, and wiping the area with a gauze sponge following each scrub. After a 5 second air drying time the needle was inserted into the vein and blood either aspirated into a syringe or vacutainer tube depending on the collection method best for the subject. A purple top tube with EDTA anticoagulant was filled

for each participant. Immediately after collection the tubes were inverted several times and placed covered on an orbital mixer for several minutes.

Whole blood for Mn analysis was refrigerated until shipped at least monthly to the New York State Dept of Health's (NYSDOH) Wadsworth Center, Health Research, Inc. Albany Division. The Laboratory of Inorganic and Nuclear Chemistry under the direction of Dr. Patrick Parsons conducted the analyses of the whole blood for Mn by Graphite Furnace Atomic Absorption Spectroscopy (GFAAS). A Perkin-Elmer Model 5100ZL Atomic Absorption Spectrometer with Zeeman background correction and equipped with an AS-70 Auto-sampler was used for the analysis of the samples. The instrument was operated using a hollow cathode lamp at the 279.5 nm line along with a slit width of 0.2 nm. Blood was diluted 1 to 9 with an aqueous solution of 0.015% $Mg(NO_3)_2$ $6H_2O$, 0.1% Triton® X-100, and 0.2% HNO_3. Using the auto-sampler twenty (20) µL of the diluent was deposited into a graphite tube onto a L'vov platform. The diluent of each sample was analyzed twice. The Method Detection Limit (MDL), defined as three times the standard deviation of twenty separate runs of a low Mn concentration blood, was 1.5 µg/L for blood Mn. Quality control consisted of incorporating numerous secondary blood reference materials into daily analytical runs. There are no certified reference materials currently available for blood Mn (Praasma et al., 2011).

Hair. Hair samples were obtained from children using ceramic scissors cleaned with alcohol. Samples were taken from the occipital region, cut close to the scalp, and deposited in clean, white envelopes. Approximately 20 strands of hair were isolated and cut. For children with very short hair, samples were taken from several areas of the head and collected directly into the envelope. Hair was analyzed for Mn concentrations (µg/g) at the Channing Trace Metals Laboratory, Brigham and Women's Hospital, Harvard School of Public Health (Boston, MA) using methods previously described (Wright et al., 2006).

Briefly, hair samples were cleaned in 10 mL of 1% Triton X-100 solution for 15 min, followed by repeated rinsing with distilled deionized water, and drying at 70 °C for 24 h. Subsequently, the samples were digested with 1 mL concentrated nitric acid for 24 h and diluted to 5 mL with deionized water. Acid-digested samples were analyzed using inductively coupled plasma-mass spectrometry (ICP-MS) (Elan DRC II, Perkin Elmer, Norwalk, CT). Analysis of Mn was performed using indium as the internal standard. Recovery of the analysis of quality control standard by this procedure is 90–110%, and the coefficient of variation of the within-day analysis was < 10%. Samples were measured by the instrument five times and the average of the five replicates is reported for each child. The average method detection limit was 1.63 ng/g.

Data analysis. The distribution of stationary and personal air Mn exposure was examined and natural log transformation was used to reach normality (ln_{sMn} and ln_{pMn} for stationary and personal Mn respectively). Geometric means, geometric standard deviations, and 95% confidence intervals were used to describe the air Mn data. The Mn and $PM_{2.5}$ concentrations of stationary and personal sampling were compared between the two testing years (2009 and 2010). Multiple linear regression models were used to examine the personal air Mn concentrations as a function of TWD, stationary air Mn concentration, wind speed and direction, and average precipitation. Separate regression models were used to examine the association between personal Mn concentration and blood and hair Mn. All data analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, NC), and a significance level of 0.05.

Study data were collected and managed using REDCap electronic data capture tools hosted at the University of Cincinnati. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common

statistical packages; and 4) procedures for importing data from external sources (Harris et al., 2009).

Results

A total of 48 children participated in the personal air sampling study. Equipment failures, i.e., air pump stopped during the sampling period, resulted in exclusion of 10 (20.8%) participants from the data analysis. Thus, complete personal air sampling data were collected on 38 children living within a 28.5 km radius of the ferromanganese refinery (Figure 1). All children were 7 (21%), 8 (39%), or 9 (39%) years old, and 66% (n=25) of the participating children were female. The majority of the participants were Caucasian (95%, n=36), 1 was African American (3%), and 1 was Native American (3%) (Table 1).

Although families were excluded based on report of a smoking family member, three (8%) participating families reported having a family member smoking in the home during the personal air sampling for 1-2 hours. Two (5%) children reported spending more than 3 hours during the 48 hour sampling period outside of the Marietta area (Table 1). The majority (n=29, 76%) remained in the Marietta area during the sampling period. The personal air sampling monitor remained with 28 (74%) of the children during the entire sampling period, whereas 7 children (18%) did not have the monitor for 1-2 hours, and 2 children (5%) reportedly did not have the monitor for 3-4 hours (Table 1).

Mean blood Mn concentration in the personal air sampling cohort was 9.5 µg/L ranging from 5 µg/L to 14.4 µg/L. Mean hair Mn concentration was 0.47 µg/g and ranged from 0.085 µg/g to 1.25 µg/g. Hair Mn and blood Mn were not significantly correlated in this sample, with correlation coefficient of 0.26, p=0.15. In multiple linear regression model adjusting for age, sex, and daily dietary Mn intake, hair Mn was only slightly higher in persons with high blood Mn with

an estimate of 0.05 µg/g increase in hair Mn per 1 µg/L blood Mn (95% CI: -0.001 to 0.095, p=0.06). Approximately 58% (22/38) of the children had their blood and hair collected within 14 days of their participation in the personal air sampling component of the study.

Personal air Mn concentration varied between the 2009 and 2010 during Spring sampling periods (Table 2). The personal air Mn appeared to be higher in 2010, but the variation did not reach statistical significance. The stationary air Mn also demonstrated similar trend, but again no statistical significance was found between 2009 and 2010. Differences between stationary and personal air Mn concentrations were not significant in both 2009 and 2010.

$PM_{2.5}$ concentration was consistently higher in the personal air filters than the stationary air filters ($p<0.05$ for both 2009 and 2010). The stationary air PM2.5 concentration was slightly higher in 2010 than 2009, with borderline significance ($p=0.06$).

When combining the sampling periods for those with personal air sampling (n=38), the geometric mean (GM) personal air Mn was 8.1 ng/m³, and the GM stationary air Mn was 11 ng/m³ (Table 3). The mean stationary $PM_{2.5}$ was 10.8 µg/m³, and the GM personal $PM_{2.5}$ was 16.9 µg/m³. The TWD ranged from 4.7 km to 28.5 km with a mean distance of 11.1 km (4.7 km st.dev) from the ferromanganese refinery (Table 3). During the entire sampling period, wind speed averaged 8.1 km/hr and ranged from 2.6 to approximately 15 km/hr, and it rained an average of 0.43 cm (st.dev 0.54 cm; range 0 – 2.25 cm). The amount of time the participating children spent outdoors ranged from 0% to 45% with an average of 14% (st.dev.12%).

Based on the multiple regression model, personal air Mn concentration (pMN) was positively associated with stationary Mn concentration and inversely associated TWD and wind speed (Table 4). For each km increase in TWD, i.e., further far from the stationary source, the ln_{pMn} was decreased by -0.075 (95% CI: -0.13, -0.01). This was roughly translated to about 8%

decrease ($1-e^{-0.075}$) in original personal air Mn concentrations by each km TWD. Neither age nor gender was associated with personal air Mn concentration.

Using separate multiple regression models, neither blood Mn nor hair Mn was associated with the stationary or personal air Mn concentrations or TWD (data not shown); however, there was a positive trend in blood Mn by personal air Mn concentration, although this was not significant (Figure 2). After repeating the analysis with only those children (n=22) whose blood and hair were collected within 14 days of the personal air sampling, there remained no statistically significant association between personal air Mn and blood or hair Mn.

Discussion

This is to our knowledge the first study of children's personal exposure to ambient Mn and its relationship with biological markers of exposure. The results of this analysis demonstrate that in this rural Appalachian community personal exposure to Mn is significantly associated with children's proximity to a ferromanganese refinery. Ambient background concentrations of Mn and wind speed were also significantly associated with personal Mn exposure. Biological markers of exposure including concentrations of Mn in blood and hair, however, were not associated with either sampled personal Mn exposure or the child's time-weighted distance from the ferromanganese facility. This can potentially be explained by the small number of children participating in the personal air sampling (n=38).

Low-level exposure to Mn has previously been associated with adverse child neurodevelopment including measures of intelligence, hyperactive behavior, memory, and motor function (Riojas-Rodrigues et al., 2010; Rodriguez-Agudelo et al., 2006; Wright et al., 2006). Similar to other environmental neurotoxicants, exposure during critical periods of neurodevelopment, including prenatally and during early-childhood, is likely to have the greatest health impact. Unlike lead and some other known neurotoxicants (e.g. second-hand smoke),

however, Mn is an essential element necessary for cellular function (Aschner and Aschner, 2005). Inhalation is a particularly significant route of exposure to Mn as this pathway bypasses homeostatic excretory mechanisms allowing for direct exposure to the lung and brain via the olfactory nerve (Dorman et al. 2002; Tjalve et al. 1996). The significance of this study is underscored by its focus on children, who are considered particularly vulnerable to environmental neurotoxicants (National Research Council 1993; Woodruff et al., 2004; Landrigan et al., 2011).

Similar to other studies of children's personal exposure to $PM_{2.5}$, our cohort's personal exposure to $PM_{2.5}$ was elevated in comparison to concurrent stationary air sampling. This observation, frequently referred to as a child's 'personal dust cloud', is likely a function of the time-activity patterns of children, including time spent indoors at home and school, in vehicles, or walking, running or playing resulting in the resuspension of particles (Ozkaynak et al., 1996; Elgethun et al., 2003). Interestingly, however, personal exposure to Mn did not follow this trend across sampling periods. Whereas in the 2009 sampling period, personal Mn exposure did, on average, exceed Mn concentrations at the stationary sampling site, the results of the 2010 personal air sampling demonstrated decreased personal Mn exposure compared to the stationary site. These findings may be a result of differing meteorological conditions, elevation of participant residences, or alterations in Mn emissions from the facility. In contrast to other studies of personal $PM_{2.5}$ exposure, the variability in personal Mn exposure was similar, and in 2010 less than, the observed variability in stationary air sampling. These results suggest that resuspension of particles is not a significant source of Mn exposure; rather, as seen by the association between distance to the ferromanganese facility and personal exposure, location near the industrial point source is likely the most significant source of personal exposure.

Though the association between personal exposure to ambient Mn and concentrations of Mn in hair were not statistically significant, we did observe a borderline relationship between

personal exposure and blood Mn. Whereas hair Mn may reflect longer term exposure, concentrations of Mn in blood are likely to reflect more recent exposures due to its short half-life (Smith et al., 2007; Zheng, 2000). The relationship between personal Mn exposure and blood Mn is complicated by the large individual variability of blood Mn due to diet, iron status, and genes involved in iron metabolism (Haynes et al 2010; Claus Henn et al 2011). However, the concentrations of Mn in blood observed in this study were similar to or greater than other studies of children with environmental exposure to ambient Mn. For example, children residing near Mn mining and processing facilities in Mexico were reported to have a geometric mean Mn blood concentration of 9.71 µg/L (Riojas-Rodriguez et al., 2010). A study of children ages 6-12 residing near a ferromanganese alloy plant in Brazil also reported a mean blood Mn concentration of 8.2 µg/L.

Hair Mn in this study is at least 10-fold lower than was measured in similar cross-sectional studies of children in Brazil where mean hair Mn was 5.8 µg/g ranging from (0.1 to 86.7 µg/g) (Menezes-Filho et al., 2011), in Mexico where the hair Mn ranged from 0.40 to 48 µg/g (Hernández-Bonilla et al., 2011), and in children exposed to Mn in drinking water in Canada (Bouchard et al., 2007) where the mean hair Mn was 5.1 µg/g. Our results, however, more closely approximate the measured Mn in hair of children residing near a hazardous waste site in Oklahoma (Wright et al., 2006) where mean hair Mn was 0.47 µg/g ranging from 0.89 to 2.1 µg/g and a study of young adults in Poland (Chojnacka et al 2010) where mean hair Mn was 0.73 µg/g . Variation in hair sample length used and analysis methods may contribute to differences in hair Mn concentration. Similar to our study, Wright et al. (2006) analyzed their samples by ICP-MS and used the entire hair sample collected, whereas Menez-Filho et al., (2011), Bouchard et al., (2007) and Hernández-Bonilla et al., (2011)used Zeeman Atomic Absorption Spectroscopy and reported using only the first centimeter or the amount available. Although human scalp hair has been used for decades to assess environmental exposure to

metals (Kopito et al., 1967; Hammer et al., 1971; Yamaguchi et al., 1971; Klevay, 1973; Menezes-Filho et al., 2011) variation across studies in the location of the hair sample collected, washing procedure, and length of hair analyzed (ATSDR, 2003, Seidel et al., 2001) limit the ability to compare hair Mn across studies. Thus, inter- and intra-laboratory validation of hair Mn collection, cleaning and analysis are needed.

A potential limitation of the current study is the number of study subjects. Due to the difficulty associated with personal air sampling in children, a subset of the overall CARES cohort was enrolled in this study. The objective of this study, however, was to determine if a surrogate of Mn exposure, time-weighted distance to the ferromanganese facility, was applicable in the general cohort. An additional limitation of this study is the lack of residential locations near the industrial point source of Mn emissions. On average, the time-weighted distance from the facility was 11.24 km ranging from 4.7 to 28.5 km. However, due to the population distribution we expect the subset to reflect the overall exposure in the CARES cohort. We are not able to assess seasonal differences in personal exposure or long term personal exposure due to sampling occurring over the course of one season. We did not observe a significant association between wind direction, as reflected by the wind index, and personal exposure to manganese. This finding may be due to the use of wind direction reported at a nearby airport as a surrogate for wind direction at the participants' homes as the topography of the study region may result in varying local wind patterns not reflected by the average regional wind direction.

In conclusion, the findings of this study suggest the time-weighted average distance from the ferromanganese facility may be a suitable surrogate of personal exposure to airborne Mn for participants in the CARES cohort.